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Human Papillomavirus Genotype Spectrum in Czech Women: Correlation of HPV DNA Presence With Antibodies Against HPV-16, 18, and 33 Virus-Like Particles

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Because the biological spectrum of human papillomavirus (HPV) genotypes present in cervical cancer lesions varies according to the geographical region studied, and because little genotype information is available for Central and Eastern European countries, we studied the endemic HPV-genotype spectrum in cervical samples collected from women visiting gynaecological departments of selected hospitals in the Czech Republic. In a series of 389 samples, 171 (44.0%) were positive for HPV DNA using a consensus-primer polymerase chain reaction (PCR). Genotyping of the HPV PCR products was done using dot-blot hybridisation with type-specific oligonucleotide probes and thermocycle DNA sequencing. Twenty-two different HPV types were detected, HPV-16 being the most prevalent type irrespective of severity of the lesions (55.0%). Multiple HPV types were found in 16.4% of our HPV-DNA-positive samples. The prevalence of HPV infection was 23.0% in women with normal findings and 59.4% in patients with cervical neoplasia, and increased significantly with the severity of the disease: 52.9% in low-grade lesions, 58.0% in high-grade lesions, and 73.5% in cervical carcinomas (P for trend < .00001). In the sera of 191 subjects, 89 with normal findings and 102 with different forms of cervical neoplasia, the prevalence of HPV-specific IgG antibodies was tested by an enzyme-linked immunosorbent assay (ELISA) using virus-like particles (VLPs) of HPV-16, -18, and -33. Antibodies were significantly more prevalent in HPV-DNA-positive than in HPV-DNA-negative women and there was no association with age. In agreement with the results of HPV genotyping, antibodies reactive with HPV-16 VLPs were the most frequent and, moreover, their prevalence increased with the cervical lesion severity. About half of the subjects with smears in which either HPV-16 or HPV-33 DNA had been detected possessed antibodies reactive with homotypic VLPs. With HPV-18-DNA-positive subjects, however, fewer than 25% displayed homotypic antibodies. In general, subjects older than 30 years of age had antibodies reactive to HPV-specific VLPs more often than subjects younger than 30 years of age. In women with benign findings, the seropositivity to HPV-16, -18, and -33 VLPs increased with age, whereas in women with cervical neoplasia the seropositivity decreased with age. ***J. Med. Virol. 58:378–386, 1999.***

KEY WORDS: PCR; HPV genotype; virus-like particles; cervical cancer

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TABLE 1. Classification of Cytological and Histological Cervical Lesions

Classification	Normal cervix	Low-grade lesions (LGL)	High-grade lesions (HGL)	Invasive carcinoma (INCA)
Papanicolaou (PAP) cytological	PAP I, II	PAP III+	PAP III-, PAP IV	PAP V
Bethesda 1989 cytological	Normal	Low squamouse intraepithelial lesions (LSIL)	High squamouse intraepithelial lesions (HSIL)	Invasive carcinoma
Histological	Normal	Cervical intraepithelial neoplasia I (CIN I)	Cervical intraepithelial neoplasia II, III (CIN II, III)	Invasive carcinoma

INTRODUCTION

An association between certain human papillomavirus (HPV) types and the development of cervical intraepithelial neoplasia (CIN) and invasive cervical cancer (INCA) is now well established [zur Hausen, 1991]. More than 100 different HPV genotypes are grouped as associated with benign (low-risk [LR] HPV) or malignant (high-risk [HR] HPV) lesions of cutaneous or mucosal epithelia [Van Ranst et al., 1992; zur Hausen, 1996]. The incidence of cervical carcinoma in the Czech Republic (22 per 100,000) is almost twice as high as in Western European countries [Brinton, 1992; Parkin et al., 1993]. An extensive World Health Organization study in 22 countries revealed that the biological spectrum of HPV types in cervical cancer varies according to geographical region [Bosch et al., 1995]. However, there is little information on the endemic HPV type-specific prevalence in Central and Eastern European countries [Steele and Gallimore, 1990; Bosch et al., 1995; Grce et al., 1997].

About 20–40% of cytologically normal women are infected with HPV [Bauer et al., 1991, 1993; Ley et al., 1991; Melkert et al., 1993]. Increasing prevalence rates and a decreasing heterogeneity of HPV genotypes have been observed over the range from low-grade cervical lesions (LGL) to INCA. In high-grade lesions (HGL) and INCA, HR HPV types have been found almost exclusively [van den Brule et al., 1990, 1991; Lorincz et al., 1992]. Depending on the method used for HPV detection, HPV DNA has been found in 35–100% of CIN [IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 1995] and in 93–100% of cervical carcinomas [Bosch et al., 1995; Jacobs et al., 1997].

The preparation of virus-like particles (VLPs) using the baculovirus recombinant system has led to significant advances in HPV serology. VLPs are indistinguishable by electron microscopy from authentic virions and they carry conformational epitopes involved in the immunological response [Steele and Gallimore, 1990; Carter et al., 1993; Hagensee and Galloway, 1993; Rose et al., 1993; Christensen et al., 1994]. A significant proportion of infected subjects are seropositive for the respective HPV types found to be present [Rose et al., 1990; Carter et al., 1993, 1994; Kirnbauer et al., 1994; Nonnenmacher et al., 1995]. Recently, it was shown that in the general population of the Czech Republic, the prevalence of antibodies to HPV-16, HPV-18, and HPV-33 VLPs increased with age [Hamšíková et al., 1998].

In the present study in Czech women, cervical samples were examined with a consensus-primer-mediated polymerase chain reaction (PCR), which allows not only the detection of all known but also novel, as yet unidentified, genital HPV types [Manos et al., 1989; Tachezy et al., 1994]. The HPV genotyping was done by hybridisation with type-specific oligonucleotide probes and by thermocycle DNA sequencing. The presence of HPV-specific antibodies in sera of our subjects was tested by enzyme-linked immunosorbent assays (ELISA) using VLPs mimicking HPV-16, -18, and -33 virions as antigens [Hamšíková et al., 1998].

MATERIALS AND METHODS

Population Studied

Cervical scrapes and/or biopsies were collected from 419 and blood samples from 191 women visiting gynaecological departments of selected hospitals in the Czech Republic. Clinical information about the patients was obtained only after the detection and typing of HPV had been completed. Thirty samples could not be amplified with β-globin primers (see below) and were therefore excluded from the study. Based on the cytological and histological findings, the remaining 389 women were divided into four groups: 165 with benign findings; 87 with low-grade lesions (PAP III+, low-grade squamous intraepithelial lesions, CIN I); 88 patients with high-grade lesions (PAP III-, PAP IV, high-grade squamous intraepithelial lesions, CIN II-III, carcinoma *in situ* [CIS]); and 49 patients with invasive carcinoma of the *cervix uteri* (INCA). The mean age of the women in our study population was 35.9 years (range 19–83 years). The mean age was 32.4 years (range 20–77 years) in the group with normal findings, 32.9 years (range 19–53 years) in women with LGL, 39.4 years (range 22–77 years) in women with HGL, and 44.8 years (30–83 years) in the INCA patients. For some patients, only cytological results and for some of the others only histopathological findings were available. The criteria for dividing the subjects into four groups are summarised in Table I. In rare instances with disagreement between histopathological and cytological results, we relied on the histopathological finding in assigning the patient into one of the catego-

ries. A total of 335 scrapes and 54 biopsies were investigated. The proportion of scrapes versus biopsies was 159 (96.4%)/6 (3.6%) in women with benign findings and 176 (78.6%)/48 (21.4%) in women with cervical lesions.

DNA Extraction

Cervical brushes were inserted in tubes with transport medium (phosphate-buffered saline [PBS] with 5 mM ethylenediamine tetraacetic acid [EDTA], pH 8.0) and stored at +4°C. The tubes were vortexed and cells were collected by centrifugation at 1,000 rpm for 10 min. Forty microlitres of each cell pellet was digested in 100 μl of lysis buffer (50 mM Tris-HCl, pH 8.0, 1% Laureth-12, 5 mM EDTA, pH 8.0) containing 100 μg/ml proteinase K (Sigma, St. Louis, MO) and incubated for 2 hr at 55°C. Proteinase K was inactivated for 10 min at 95°C. Samples were stored at –20°C. Biopsy samples were cut on dry ice and incubated at 37°C overnight in 50 mM Tris-HCl, pH 8.6, 50 mM EDTA, pH 8.0, 0.5% sodium dodecyl sulphate (SDS) and 0.5 mg/ml proteinase K. Genomic DNA was extracted from tissue lysate by a nonphenol extraction procedure [Miller et al., 1988], precipitated with 2 volumes of absolute ethanol, air-dried, and resuspended in 10 mM Tris-HCl, pH 8.0. The DNA samples were stored at –20°C.

Detection of HPV by PCR

PCR was carried out using consensus primers (MY09 and MY11) to a highly conserved region in the L1 open reading frame of HPV DNA. Briefly, 100 μl of the reaction mixture consisted of 10 mM Tris-HCl, pH 8.5, 50 mM KCl, 4 mM $MgCl_2$, 0.2 mM each of the four dNTPs (Promega, Madison, WI), 0.5 pmol each of the MY09 and MY11 primers, and 2.5 U Taq polymerase (Promega). A control primer set (PC04 and GH20, 0.05 pmol each), which amplified a 268-bp β-globin gene fragment, was included in the reaction to ensure the presence of an adequate amount of amplifiable DNA in the sample. Negative controls without template and with HPV-negative DNA of LEP cells, a human lung embryonic fibroblast cell line, were included to detect possible carry-over contamination. DNA from SiHa, a human cervical cancer cell line containing one to two copies of the HPV-16 genome per cell, was used as a positive control.

Each sample was subjected to 35 cycles of amplification in a Gene E thermocycler (Techne, Cambridge, UK) as follows: 95°C for 1 min, 55°C for 2 min, and 72°C for 2 min. The first cycle was preceded by 5-min denaturation at 95°C, and the last cycle was followed by incubation at 72°C for 3 min. An aliquot of 10 μl from each amplification reaction was separated electrophoretically on a 3% agarose gel (NuSieve 3:1) (FMC BioProducts, Rockland, ME), stained with ethidium bromide, and visualised by fluorescence under ultraviolet (UV) light. After 10 min of initial depurination of the gel in 0.25 N HCl and equilibration in an alkaline transfer solution (0.6 M NaCl, 0.4 N NaOH) for 15 min, Southern blot transfer was carried out to a Biodyne B nylon membrane (Pall Biosupport, Glen Cove, NY) using the alkaline transfer method. The membrane was neutralised for 15 min in 1 M Tris-HCl (pH 7.4) and 1.2 M NaCl, and the DNA was fixed to the membrane by baking at 80°C for 2 hr. The hybridisation was done overnight at 42°C in 5× SSC, 50 mM $NaPO_4$, 0.1% SDS, and 10× Denhardt solution, with a mixture of 410-bp long radioactive probes. The probes had been PCR generated and labelled using type-specific nested primers for HPV-11, -16, -18, and -51 in reactions containing 5′-[α^{32}P]-dCTP. A 20-bp oligonucleotide β-globin probe (PC03) was end-labelled with T4 polynucleotide kinase (USB, Cleveland, OH). The membranes were washed twice for 30 min at 50°C under low stringency conditions (2× SSC, 0.1% SDS). The sensitivity of our PCR followed by hybridisation was estimated to be 1–10 copies of viral genome per reaction using a dilution series of HPV-16 containing SiHa cells in the presence of an excess of HPV-negative LEP cells.

Typing of HPV by Dot-Blot Hybridisation

Three microlitres of each sample that showed the 450-bp HPV-specific band after initial PCR and hybridisation were dot-blotted on six identical Biodyne B nylon membranes (Pall Biosupport). Prehybridisation was done at 65°C for 15 min in 5× SSPE and 0.1% SDS. Each of the six membranes (A–F) was hybridised with a mixture of type-specific oligonucleotide probes at 50°C overnight. Membrane A: HPV-6, 11, 42, 57; membrane B: HPV-16; membrane C: HPV-18; membrane D: HPV-54, 55, 56, 59, 53; membrane E: HPV-39, 45, 51; membrane F: HPV-31, 33, 35, 52, 58. The membranes were washed at 56°C in 2× SSPE and 0.1% SDS. The samples positive after hybridisation with one or more mixtures of type-specific oligonucleotide probes were then hybridised with each probe of the respective mixture separately [Manos et al., 1989; Bauer et al., 1991; Hildesheim et al., 1994; Tachezy et al., 1994].

Typing of HPV by Nucleotide Sequencing

The 450 bp PCR-fragments that failed to hybridise with any of the type-specific oligonucleotide probes were cut out of the gel, purified on QIAquick Gel Extraction columns (Qiagen, Hilden, Germany) and subcloned in a pMOSBlue T-vector (Amersham Life Science, Cleveland, OH). The DNA was extracted through QIAprep Spin Miniprep columns (Qiagen) and sequenced with a Thermo Sequenase Fluorescent Labeled Primer Cycle Sequencing Kit with 7-deaza-dGTP (Amersham Life Science). The Cy-5-labelled pMOSBlue T-vector sequencing primers were prolonged with three nucleotides at their 5′ end (T7 primer: 5′-GCTCTAATACGACTCACTATAGGG-3′; U19 primer: 5′-AGGGTTTTCCCAGTCACGACG-3′). Cycling consisted of 25 cycles: 95°C for 30 sec, 65°C for 30 sec, 72°C for 30 sec. The sequencing was performed on an ALFred automatic sequenator (Amersham Pharmacia Biotech, Roosendaal, The Netherlands) in 0.6% TBE at 55°C and 1,500 V. Alternatively, PCR products were extracted from gel with β-agarase I enzyme

(USB), sequenced with a Dye Primer Cycle Sequencing Kit (Perkin Elmer, Norwalk, CT), and analysed on an ABI PRISM 310 genetic analyser (Perkin Elmer).

Sequence Analysis

DNA sequence assembly and analysis were conducted using the PC/GENE software package release 6.7 (Intelligenetics, Inc., Campbell, CA). GenBank database (release 98) searches were carried out using the BLAST server at the National Center for Biotechnology Information (NCBI; Bethesda, MD).

Serology

Sera were tested by ELISA using baculovirus-expressed L1 or L1 and L2 VLPs mimicking HPV-16, -18, and -33 virions. The baculovirus recombinants expressing the L1 of HPV-16 and HPV-18 were kindly provided by J. Schiller (NIH, Bethesda, MD) and recombinants expressing both L1 and L2 of HPV-33 were a kind gift from M. Sapp (Johannes-Gutenberg-University, Mainz, Germany). From insect cells infected by recombinant baculoviruses, the capsids were purified on CsCl gradients [Kirnbauer et al., 1993; Volpers et al., 1994]. The ELISA was carried out as described elsewhere [Hamšíková et al., 1997]. Briefly, microtiter plates were coated with about 1 μg of antigen preparation in 50 μl of PBS per well, which corresponded to 4–8 antigen units. One antigen unit was defined previously, using a mixture of human sera strongly reactive with an appropriate antigen, as the lowest amount of antigen to give a positive reaction in the presence of antibody excess. Wells were incubated at 37°C and potentially free binding sites were blocked by 0.1% fetal calf serum in 0.5% non-fat dry milk in PBS. Sera were tested at a dilution 1:25; the wells were washed then incubated with peroxidase-conjugated anti-human IgG and the reaction was visualised using 2,2′-azino-bis(3-ethylbenzthiazoline 6-sulphonic acid) diammonium salt (ABTS). The cut-off value was calculated for each antigen separately as the mean absorbance value +2 SD after eliminating outliers achieved with sera originating in HPV-DNA-negative women; before calculation, the absorbance value of antigen-free control wells was subtracted.

Statistical Analysis

The standard chi-square test and in some instances the Fischer exact test were used. Odds ratios (OR) with 95% confidence intervals (CI) and two-tailed P values were calculated in 2 × 2 tables using the EPI INFO statistical package (version 6.04). In all tests, the basic significance level was $P = .05$. Multiple logistic regression analysis was conducted using the Statistical Analysis Program SPSS-PC.

RESULTS

Cervical specimens from 419 women were tested. Thirty of the cervical specimens did not amplify with the β-globin primers used and were excluded from the study. The results are summarised in Table II. Among

TABLE II. HPV Presence in Population Studied

	No. of tested specimens	HPV positive	Total isolates	HPV type																					
				LR $n = 19$ (9.2%)				HR $n = 188$ (90.8%)																	
				6/11	54	42[a]	73	16	18	26	31	33	35	39	45	51[a]	52	53	56[a]	58	66	70	MM4	MM8
n	389	171	207	11	5	1	2	114	16	1	9	10	3	5	5	3	3	6	1	8	1	1	1	1
%		44.0		5.3	2.4	0.5	1.0	55.0	7.7	0.5	4.3	4.8	1.4	2.4	2.4	1.4	1.4	2.9	0.5	3.9	0.5	0.5	0.5	0.5

HPV, human papillomavirus; LR, low-risk HPV types; HR, high-risk HPV types.
[a]Types detected in multiple infection only.

the 389 amplifiable samples (335 cervical scrapes and 54 biopsies), 171 were HPV positive; 22 different HPV types were detected. The positivity reached 40% (134/335) for scrapes and 68.5% (37/54) for biopsies. The prevailing type was HPV-16 (55.0%), followed by HPV-18 (7.7%); HPV-6/11 (5.3%); HPV-33 (4.8%); HPV-31 (4.3%); HPV-58 (3.9%); HPV-53 (2.9%); HPV-39, -45, -54 (2.4% each); HPV-35, -51, -52 (1.4% each); HPV-73 (1.0%); and HPV-26, -42, -56, -66, -70, MM4, MM8 (0.5% each). The prevalence of HPV infection increased with the severity of the cervical lesions (Table III). In cervices with normal findings, the prevalence of HPV was 23.0%, in patients overall, 59.4%. HPV positivity reached 52.9% in LGL, 58% in HGL, and 73.5% in INCA (P for trend < .00001). The heterogeneity of HPV types decreased with severity of the disease (17, 14, 11, and 8 different HPV types in normal women, LGL, HGL, and INCA, respectively). The prevalence of HR HPV types increased significantly with disease severity (P for trend < .00001). As shown in Table IV, infection with multiple HPV types was detected in 28 specimens (16.4%), in 7/37 biopsies (18.9%), and in 21/134 scrapes (15.7%). Infection with two HPV types was found in 20 specimens, HPV-16/18 being the most frequent combination. In eight samples, three HPV types were detected; the most common combination was HPV-11/16/18. The simultaneous presence of two or more different types in HPV-positive subjects was almost equally frequent in all subject groups (15.8, 19.6, 15.7, and 13.9% of specimens positive for multiple HPV types in normal women, LGL, HGL, and INCA, respectively).

The results of serological tests with the 191 serum samples available are summarised in Tables V and VI. As shown in Table V, 36.6% of our subjects were seropositive for antibodies to one or more of the VLPs. Twenty-four percent of the subjects had antibodies to HPV-16 VLP, 19.4% to HPV-18 VLP, and 16.2% to HPV-33 VLP. We observed a higher prevalence of antibodies to HPV-specific VLP in HPV-DNA-positive than in HPV-DNA-negative women in both those with benign findings and those with cervical lesions. However, these differences were statistically significant only for HPV-33 VLP ($P = .0381$) in the group of women with normal findings and for HPV-16 VLP ($P = .0115$) in the patient group (Table VII). In general, antibodies were found more frequently in patients than in healthy subjects. However, this association was significant only for HPV-16 VLP. The prevalence of antibodies to HPV-16 VLP increased with cervical lesion severity (P for trend < .02).

The HPV genotype-specificity of the antibodies is shown in Table VI. Approximately half of the women positive for HPV-16 DNA (46.3%) or HPV-33 DNA (50.0%) possessed antibodies reactive with the respective HPV VLP. However, in many of the HPV-16 DNA-positive subjects either HPV-18 or HPV-33 antibodies were also detected. The correlation of the presence of HPV-16 or HPV-33 DNA with the presence of the respective homotypic HPV antibody was highly signifi-

TABLE III. HPV-Type Distribution in Normal Cervix and in Cervical Lesions

Clinical status	No. of tested specimens	HPV positive	Total isolates	HPV type																		
				LR $n = 19$ (9.2%)				HR $n = 188$ (90.8%)														
				6/11	42[a]	54	73	16	18	26	31	33	35	39	45	51[a]	52	53	56[a]	58	66	70	MM4	MM8
Normal cervix (n)	165	38 (23.0%)	44	3	—	2	1	17	2	—	4	2	1	2	—	1	1	2	1	2	1	—	1	1
LGL (n)	87	46 (52.9%)	60	5	1	3	—	30	4	1	2	2	2	—	1	1	1	2	—	5	—	—	—	—
HGL (n)	88	51 (58.0%)	61	2	—	—	—	38	5	—	1	6	—	1	3	1	1	2	—	—	—	1	—	—
INCA (n)	49	36 (73.5%)	41	1	—	—	1	29	5	—	2	—	—	2	1	—	—	—	—	1	—	—	—	—

HPV, human papillomavirus; LR, low-risk HPV types; HR, high-risk HPV types; LGL, low-grade lesions; HGL, high-grade lesions; INCA, invasive carcinoma.
[a]Types detected in multiple infection only.

TABLE IV. Presence of Single-Type and Multiple HPV Infection in HPV-Positive Healthy Subjects and in Cervical Neoplasia Patients

No. of HPV types detected	No. of subjects	HPV-positive subjects			
		Normal cervix (n = 38)	LGL (n = 46)	HGL (n = 51)	INCA (n = 36)
One	143	32 (84.2%)	37 (80.4%)	43 (84.3%)	31 (86.1%)
More than one	28	6 (15.8%)	9 (19.6%)	8 (15.7%)	5 (13.9%)
Two	20	6 (15.8%)	4 (8.7%)	6 (11.8%)	4 (11.1%)
Three	8	—	5 (10.9%)	2 (3.9%)	1 (2.8%)

HPV, human papillomavirus; LGL, low-grade lesions; HGL, high-grade lesions; INCA, invasive carcinoma.

cant (HPV-16 VLP: $r = 0.3788$, $P < .001$; HPV-33 VLP: $r = 0.2799$, $P = .004$). This correlation was not observed in HPV-18 DNA-positive subjects; only 3 of 13 such subjects were reactive with homotypic VLP and the correlation was weak ($r = 0.0562$, $P = .560$).

In the group of 188 women tested serologically, 101 subjects were younger than 30 years and 87 women were older than 30 years. In general, the antibodies were more often present in the older age group. In the group of women with a normal cervix, the prevalence of HPV-specific antibodies was higher among the older subjects, whereas for the patients (any woman with some abnormal cytological diagnosis) the prevalence of HPV-specific antibodies was higher among the younger subjects. However, this difference attained statistical significance only for the prevalence of HPV-16 VLP among healthy subjects. The results are summarised in Table VIII.

DISCUSSION

To prepare the ground for future diagnostic, therapeutic, and vaccination strategies, a basic knowledge of HPV prevalence and HPV genotype distribution in the populations concerned would be required. Because little is known about the HPV type-specific distribution in Central and Eastern Europe, we investigated the prevalence and spectrum of HPV types in Czech women.

Three hundred eighty-nine samples were collected for HPV genotyping (335 scrapes and 54 biopsies). The positivity for HPV DNA reached 68.5% for biopsies and 40% for scrapes, which reflects the fact that most biopsies (88.9%) were obtained from patients or from women with cytological abnormality, which were not confirmed by histology (11.1%).

Because the biopsies contain more cellular material than scrapes, more biopsies than scrapes could be expected to be positive for multiple HPV types. In our study, 28 samples were found with multiple HPV types and these multiple infections occurred nearly as frequently in biopsies as in scrapes.

Using a consensus-primer-mediated PCR method, 22 different HPV genotypes were detected. Although this method allows amplification of previously unknown genital HPV types, we did not find any such unidentified types. Since the introduction of general and consensus-primer-mediated PCR strategies in 1989, about 30 novel HPV types have been recognised. The rate of discovery of additional genital HPV types has decreased over the past few years [de Villiers, 1997]. Presumably, using amplification-based methods, the known "island" of genital HPV genotypes has been characterised to near completion.

HPV infection has been detected over a range of 3.7–44% of cytologically normal women [IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 1995]. In our cohort, HPV positivity in women with normal findings reached 23.0%. Worldwide, HPV-16 is the most prevalent type in normal cervices and is found in about 50% of INCA. HPV-18 has been recognised to be the second most common genotype in all countries except Indonesia and Algeria, where it is the most prevalent type in INCA [Bosch et al., 1995]. In our study, HPV-16 was the prevailing type in normal cervices (10.3%), and its prevalence increased steeply through LGL (34.5%) and HGL (43.2%) to INCA (59.2%). HPV type-18 was the second most prevalent type, being present in 1.2% of normal cervices and in 10.2% of INCA. In other studies from Central European countries, HPV-16 has been detected in 0–7% and HPV-18 in 0–2.3% of normal cervices, but less sensitive HPV detection methods were used in those studies [Czegledy et al., 1989, 1993; Mincheva et al., 1991]. One study that used a comparable general-primer-mediated method showed a prevalence of HPV-16 in 3.7% and HPV-18 in 1.8% of normal cervices [Veress et al., 1994].

According to the literature, HPV prevalence rates in CIN lesions range between 35% and 100% [IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 1995]. In our study, HPV prevalence in CIN was 55.4%. Our HPV positivity rates in HGL (58%) and INCA (73.5%) were lower than in the International Biological Study on Cervical Cancer (IBSCC) [Bosch et al., 1995], probably because detection in the IBSCC study was carried out exclusively on colposcopy-directed biopsies, whereas in our study, biopsies accounted for only 13.9% of the samples.

In our study, sera were obtained from 191 subjects and examined for the presence of antibodies reactive with HPV-specific VLPs. The cohort of women displayed antibodies to HPV-16, -18, and/or -33 VLPs at a joint rate of 36.6%. Seropositivity was more common among women with cervical disease than in women

TABLE V. Presence of Antibodies Against HPV-16, -18, and -33 VLPs in Relation to HPV-DNA Positivity and Clinical Status

Subjects investigated	*N*	Antibody presence									
		HPV-16			HPV-18			HPV-33		VLP any	
		n (%)	OR	AAOR	*n* (%)	OR	AAOR	*n* (%)	OR	AAOR	*n* (%)	OR	AAOR
Total	191	46 (24.0)			37 (19.4)			31 (16.2)			70 (36.6)		
HPV DNA-negative	97	12 (12.4)	1.0	1.0	16 (16.5)	1.0	1.0	10 (10.3)	1.0	1.0	26 (26.8)		
HPV DNA-positive	94	35 (36.2)	4.0***	3.8***	21 (22.3)	1.5	1.1	21 (22.3)	2.5**	2.4**	44 (46.8)	2.4**	2.2**
Normal cervix	89	13 (14.6)	1.0	1.0	13 (14.6)	1.0	1.0	13 (14.6)	1.0	1.0	26 (29.2)	1.0	1.0
LGL	28	7 (25.0)	2.0	1.9	7 (25.0)	2.0	2.0	5 (17.9)	1.3	1.3	10 (35.7)	1.4	1.4
HGL	44	13 (29.5)	2.5**	2.5*	9 (20.5)	1.5	1.0	9 (20.5)	1.5	1.2	19 (43.2)	1.8	1.7
INCA	30	13 (43.3)	4.5**	4.4**	8 (26.7)	2.1	1.1	4 (13.3)	0.9	0.6	15 (50.0)	2.4**	2.0
P for trend			<.02			.15			.79			<.02	

HPV, human papillomavirus; VLP, virus-like particle; LGL, low-grade lesions; HGL, high-grade lesions; INCA, invasive carcinoma; OR, odds ratio; AAOR, age-adjusted odds ratio.
**$P < .05$.
***$P < .001$.

with normal findings, and the prevalence of antibodies to HPV-16 VLP increased with cervical-lesion severity. Antibodies against HPV-33 VLP were more frequent in the HGL than in the INCA group of women. Similar results have been reported by Wideroff et al. [1995] and Nonnenmacher et al. [1996].

The seroprevalence of antibodies to HPV-16, -18, or -33 VLP was significantly higher in HPV-DNA-positive than in HPV-DNA-negative women in our study, and this difference was significant for HPV-16 and -33 VLPs. Additionally, antibodies to HPV-16, -18, -33 VLP were present more often in HPV-DNA-positive than in HPV-DNA-negative subjects when the groups of normal women and patients were evaluated independently. However, the difference was statistically significant only for HPV-16 VLP in the patients group and for HPV-33 VLP in the group of women with a normal cervix.

The seroprevalence of antibodies against HPV-16 VLP was 46.3% in the HPV-16-DNA-positive group, and 12.4% in the HPV DNA-negative group. Similar HPV-16-VLP seroprevalence values in HPV-16-DNA-positive subjects have been detected in other studies [Christensen et al., 1994; Kirnbauer et al., 1994; Nonnenmacher et al., 1995], but some investigations have failed to find a significant difference in HPV-16-VLP seroprevalence between HPV-16-DNA-positive and HPV-DNA-negative subjects [Nonnenmacher et al., 1996].

In the HPV-18-DNA-positive group, 30.8% of sera also reacted with HPV-16 VLP. This finding can be explained by prior, or undetected concurrent, infection with HPV-16, the most prevalent HPV type. It is noteworthy that women with HPV-31 or -33 DNA in the cervix showed no such reactivity with HPV-16 VLP (see Table VI). Similarly, Kirnbauer et al. [1994] had shown that 31% of their HPV-18-DNA-positive women had antibodies to HPV-16 VLP, but in their study 38% of sera obtained from HPV-31-DNA-positive women also reacted with HPV-16 VLP. The present results are in good agreement with a previous report, in which 50% of HPV-33-DNA-positive Spanish or Colombian patients reacted with HPV-33 VLP [Hamšíková et al., 1997]. It is remarkable that among our HPV-18-DNA-positive subjects, HPV-18 VLP antibodies were less frequent than among HPV-16-DNA-positive women. A lower immunogenicity of HPV-18 might be one of the alternative explanations for this discrepancy.

Recently published data show that the prevalence of antibodies to HPV in the general healthy population increases with age [Hamšíková et al., 1998]. Also, in our study, the subjects older than 30 years were seropositive more frequently than the subjects younger than 30 years of age in the group of women with normal cervix. In contrast, in the patients group the reverse was true.

Our study supports the assumption that the detection of antibodies against HPV VLPs can be explored as an albeit imperfect surrogate marker of HPV infection. Interpreting a positive result on serological testing for

TABLE VI. Type Specificity of Antibodies Against HPV-16, -18, and -33 VLPs

HPV DNA detected	*N*	Antibody presence: HPV-16 *n* (%)	HPV-16 OR	HPV-18 *n* (%)	HPV-18 OR	HPV-33 *n* (%)	HPV-33 OR
None	97	12 (12.4)	1.0	16 (16.5)	1.0	10 (10.3)	1.0
16	67	31 (46.3)	6.1***	14 (20.9)	1.3	12 (17.9)	1.9
18	13	4 (30.8)	3.2	3 (23.1)	1.5	3 (23.1)	2.6
33	6	0 (—)	—	1 (16.7)	1.0	3 (50.0)	8.7**
31	5	0 (—)	—	1 (20.0)	1.3	1 (20.0)	2.6

HPV, human papillomavirus; VLP, virus-like particle; OR, odds ratio.
**$P < .05$.
***$P < .001$.

TABLE VII. Relationship Between Antibody Presence, HPV DNA Presence, and Clinical Status

Clinical status	HPV DNA	*N*	Antibody presence: HPV-16 *n* (%)	HPV-16 OR	HPV-16 *P*	HPV-18 *n* (%)	HPV-18 OR	HPV-18 *P*	HPV-33 *n* (%)	HPV-33 OR	HPV-33 *P*
Normal cervix	Negative	68	8 (11.8)	2.3	.170	9 (13.2)	1.5	.510	7 (10.3)	3.4	.038**
	Positive	21	5 (23.8)			4 (19.0)			6 (28.6)		
Patients	Negative	29	4 (13.8)	4.1	.011**	7 (24.1)	1.0	.927	3 (10.3)	2.2	.220
	Positive	73	29 (39.7)			17 (23.3)			15 (20.5)		

HPV, human papillomavirus; OR, odds ratio.
**$P < .05$.

TABLE VIII. Relationship Between Antibody Presence and Age in Women with Normal Findings and in Patients

Clinical status	Age group	*N*	HPV-16 *n*	HPV-16 *n* (%)	HPV-16 OR	HPV-16 *P*	HPV-18 *n*	HPV-18 *n* (%)	HPV-18 OR	HPV-18 *P*	HPV-33 *n*	HPV-33 *n* (%)	HPV-33 OR	HPV-33 *P*	VLP any *n*	VLP any *n* (%)	VLP any OR	VLP any *P*
Normal cervix	<30	62	6	9.7	3.3	.047**	7	11.3	2.2	.182	7	11.3	2.2	.182	16	25.8	1.7	.287
	>30	27	7	25.9			6	22.2			6	22.2			10	37.0		
Patients	<30	25	12	48.0	2.5	.054	8	32.0	0.5	.129	6	24.0	0.5	.220	14	56.0	0.5	.088
	>30	74	20	27.0			13	17.6			10	13.5			27	36.5		

HPV, human papillomavirus; OR, odds ratio.
**$P < .05$.

an almost ubiquitous virus such as HPV must take into account that this test result represents the serological relicts of all past and present, clinical and subclinical, acute or persistent HPV encounters.

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